APPENDIX B: SUMMARY OF INFORMATION COLLECTED FROM TELEPHONE CONVERSATIONS WITH INDUSTRY REPRESENTATIVES AND UNIVERSITY RESEARCHERS

In 1987, ICF designed and conducted a survey of biotechnology companies potentially subject to regulatory reporting requirements under TSCA (ICF 1988). Between 1987 and 1991, the industry and the related expectations of growth changed significantly. To update the survey and provide a more current estimation of activities potentially subject to TSCA, in 1991, ICF assisted the Regulatory Impacts Branch, Economics, Exposure, and Technology Division of the U.S. Environmental Protection Agency by recontacting a portion of the industry and assessing their current and planned activities. In addition, answers to questions that addressed information outside the scope of the 1988 survey were sought. The remainder of this appendix addresses the issues covered during the telephone conversations which took place in 1991.

During the follow-up, a number of researchers, representatives, and other experts at various companies, universities, trade associations, and other organizations were contacted by ICF in order to develop, informally, a consensus of information regarding, among other topics, basic research, commercialization plans, monitoring and control costs, industry growth trends and TSCA market areas. The findings presented below summarize the important conclusions developed in this information gathering process.

- Section A presents information on the level and progress of activities in TSCA market areas.
- Section B presents descriptions and cost information for field monitoring, containment and control procedures that have been used.

A. Trends in TSCA Market Participation

Of the thirteen companies recontacted by ICF between the original survey in 1987 and the second survey in 1991, four were no longer active in TSCA market areas. Two of these companies decided to focus on pharmaceutical development because it was more suited to their technology (Alpha Beta

Technology 1991), or it required fewer resources and lead time than the TSCA market areas (Cetus Corporation 1991). Another company dropped activities with microorganisms in the agricultural TSCA market area because of an apparent lack of a significant market opportunity and because of technical problems (Biotechnica, Plant Research Corporation 1991). At the time of the survey, the fourth company had ceased activities with microorganisms and was importing biochemicals for distribution in the U.S. (P&S Biochemicals 1991).

One significant trend among the companies that responded to the survey in 1991 was the use of naturally occurring microorganisms in research and development instead of genetically engineered microorganisms. Five of the remaining nine companies worked predominantly with naturally occurring microorganisms. One overriding concern resulting in this trend is the desire to get products commercialized as soon as possible. Although companies do conduct research with genetically engineered microorganisms, the lag time from conception to commercialization of products may, in some cases, be too great to justify the use of rDNA techniques (Celgene 1991). Another reason for developing products with naturally occurring microorganisms instead of genetically engineered ones is to avoid the regulatory and client acceptance problems associated with genetically engineered microorganisms (Envirogen 1991a). Genetically engineered microorganisms also are more expensive to develop. Development costs for microorganisms engineered with recombinant techniques for environmental applications are likely to be on the high end of a $500,000 to $3 million a year range. These costs may overburden a small company's budget (D. Glass Associates 1991a, 1991b).

1. Fermentation-system Markets

For fermentation-system uses of microorganisms, such as in specialty chemical production, often the majority of the microorganisms in R&D are naturally occurring. In some cases, products may be genetically

engineered before commercialization to increase their commercial viability (Novo Laboratories 1991).

At the time the survey was conducted, the specialty chemical market area appeared to be growing rapidly. Eight companies indicated activity in this area and all involved processes that take place in a fermentation-system. One industry contact suggested growth in the production of industrial enzymes would be more gradual because the industry is mature (International Bio-synthetics 1991). In contrast, a spokeswoman for another company indicated the manufacturing of industrial enzymes using recombinant microorganisms is more precise and easier, so significant growth in the specialty chemical production market can be expected. Although she expected an increasing number of microorganisms to be used, she also predicted most of them would be considered exempt from TSCA regulations (Genencor International 1991). Two other contacts whose companies were active in specialty chemical production using microorganisms maintained that the market was experiencing good growth in this area (Novo Laboratories 1991, Promega 1991.) One industry representative volunteered an annual growth rate of between 10 percent and 15 percent (Promega 1991).

Other fermentation-system markets have showed slower growth and no significant shift in technologies since 1987. An industry trade association representative indicated that he expected this steady state to continue for at least another 5 years and noted that the majority of work would continue to be with naturally occurring microorganisms (IBA 1991).

2. Environmental Application Markets

Environmental application markets appear to be moving much more slowly than the fermentation-systems (IBA 1991). For example, the agriculture market area appeared to have become stagnant as illustrated by the following information:

- Biotechnica International's research and development subsidiary, Plant Science Research is no longer using microorganisms in any of its activities because technical problems were encountered and the market opportunity for products was limited (Biotechnica, Plant Science Research 1991). Biotechnica used to be very active in the agricultural TSCA market area and performed extensive field testing of the microorganisms a few years ago (D. Glass Associates 1991).

- Another company sold its agriculture division and decided to concentrate on pharmaceutical production. The spokesman for this company maintained there is still great long term potential in the agriculture TSCA market area but that the company thought its resources would be better used elsewhere (Cetus Corporation 1991).
- Other companies that continued to be active in research for the agriculture market indicated the R&D programs in this area were making slow progress (Surax 1991, Urbana Laboratories 1991).

- A representative of a trade association indicated that his impression was that most significant progress in this area was at least 10 years away and that researchers had backed away from the use of recombinant techniques because of increased development costs (IBA 1991).

In the mining/metal recovery market area three companies that were once active have discontinued their R&D programs in this area. One contact said that the company was not competitive in this area (Novo Laboratories 1991). The remaining two companies found the research and development costs associated in the metal/mining recovery TSCA market area were prohibitive (Alpha Beta Technology Company 1991, Surax Incorporated 1991). A trade association representative indicated that while none of the companies affiliated with his organization were working in this area, he had heard that some research was being conducted by other groups (IBA 1991).

Despite hindrances encountered in the TSCA market areas that commonly involve environmental applications, some companies appear to be optimistic about the opportunities for these uses of microorganisms, particularly in the waste/pollutant degradation TSCA market area. Two companies had initiated R&D projects in this area, but they did not have actual products in the laboratory yet (International Bio-synthetics 1991, Promega 1991). Four of the companies

had intact R&D programs in the waste/pollutant degradation or reclamation TSCA market areas (Celgene 1991, Envirogen 1991b, Genencor 1991, Monsanto 1991b) and three of them work almost exclusively with naturally occurring microorganisms (Celgene 1991, Genencor 1991, Monsanto 1991b).

3. Product Development

Fermentation-system and environmental applications also appear to have different product development paths. Estimates of the length of time an environmental application product would be in R&D before a field test ranged from as little as 6 months to between 2 to 5 years (Celgene 1991, Envirogen 1991, D. Glass Associates 1991b). The estimated number of field tests usually required for a single product ranged from two to four (Celgene 1991, Envirogen 1991). In both cases, microorganisms engineered using recombinant techniques were expected to be at the high ends of these ranges.

In contrast, the approximate length of time a fermentation-system product is in R&D before a pilot scale fermentation is performed can be only 3 to 4 months according to one contact (International Biotechnologies 1991). Other companies plan for about a year of R&D per product (Promega 1991, Novo Labs 1991). All of these companies agreed that these periods would be significantly longer if they were using more genetically engineered microorganisms, due to the greater complexity of these microorganisms. Several companies indicated that because a high percentage of the microorganisms they used are naturally occurring, they expected between 60 to 75 percent of their products to reach commercialization (Promega 1991, Novo Labs 1991, International Biotechnologies 1991).

4. Regulatory Factors' Influence on Research Decisions

One company spokesperson related that the anticipation of regulations dictated aspects of research on genetically engineered microbial bioremediation products. The company considered the EPA's response as an

important factor in making research decisions. In order to receive EPA approval of the product, the company might choose more expensive or less effective options for development. In anticipation of regulatory hurdles, the company also hired a regulatory consultant to provide assistance for a planned field reactor test. The spokesperson estimated that as much as half of the total development costs for genetically engineered microorganisms designed for environmental application could be attributed to the anticipation of regulations (Envirogen 1991b).

B. Monitoring, Containment, and Control Procedures

Some monitoring, containment, and control activities are inherent in all research with microorganisms (these activities are undertaken to prevent the subject microorganisms from escaping beyond the test site or from becoming contaminated by external forces not included in the experiment). These actions are necessary to develop a cause and effect relationship between the microorganism and the target substrate, and to develop a better understanding of the colonization and persistence of the organism in the environment (Lamar 1991). NIH guidelines are usually followed to ensure the safety and health of the scientists and others that could be exposed to the experimental microorganism. In many cases, NSF support entails that proper monitoring, control, and containment procedures are followed (Chakrabarty 1991).

1. Field Tests of Environmental Applications

For many environmental applications, particularly academic research, the degree of containment and control often is limited by the funds available for a field test (Parke 1991, Kline 1991, Chakrabarty 1991, Lommel 1991). The degree of control and containment for a field test will usually vary with the type of organism released, as well as with the size of the test and the environment. For example, field test sites that are exposed to heavy rainfall or wind, that are sloped rather than level, or that are close to

groundwater sources require more careful monitoring (National Research Council 1989). Organisms that have been widely studied in the laboratory generally have more predictable behavior and could entail less monitoring and control during the release. For example, according to Professor Triplett of the University of Wisconsin, the EPA and USDA decided not to impose restrictions on his experiment after assessing monitoring and containment procedures. He stated that EPA and USDA found that Rhizobium has been extensively studied and is considered safe. The Institutional Biosafety Committee at Wisconsin also agreed to the procedures (Triplett 1991).

Researchers contacted generally felt that work with genetically modified microorganisms would require careful monitoring and control, both in the laboratory and for the eventual release. Dr. Ellis Kline of Clemson University has worked on two field tests of genetically engineered microorganisms that were funded by Monsanto. He stated that the field tests (especially the first test) required monitoring and control to closely examine the growth and dispersion of the microorganism and its effect on the environment and human health and safety. He also outlined the basic method of monitoring that he felt should be used.

The general methods of containment and monitoring that Dr. Kline felt should be used involve the following: the development of a baseline in the laboratory using well controlled systems, the use of a logical delivery system, conducting toxicology studies including development of acceptable LD 50s for two species of animals, pathogenic plant analyses, and other toxicological tests such as Daphnia tests. Monitoring can focus on the microorganism's persistence, vertical and horizontal dispersal, lateral migration, air-borne migration, and surface water runoff. Important questions that should be examined include:

Does it colonize?
Does the colony reach equilibrium over time?
Does the colony reburst with reintroduction of stimuli?

Kline stated that efforts for monitoring could be consolidated after further study and for well studied organisms. Although they deal with applications outside TSCA market areas, the following examples illustrate some procedures that have been used to monitor field tests.

- Professor Steven Lindow of the University of California at Berkeley conducted the first FIFRA-approved field test of recombinant microorganisms in the United States. EPA required that an Experimental Use Permit (EUP) be obtained prior to release. FIFRA requirements on information provision and documentation prior to the release and monitoring procedures required during the release were extremely stringent and costly. Documentation and monitoring costs for field tests today under FIFRA are substantially less (Lindow 1991).

  Before the release of the recombinant bacteria, laboratory and greenhouse tests were done to document safety to human health and the environment. Greenhouse studies were conducted measuring the competitiveness, habitat preferences, and behavior of ice-minus in relation to ice-plus strains of P. syringae. Experiments were also done measuring the dispersal of P. syringae during and after inoculation.

  To contain the organism, a weed free area surrounding the inoculated plot separated any other crops from the treated plants by at least 30 meters (P. syringae does not survive in soil).

  Monitoring took several forms. First, aerosol plumes were monitored for dispersal of bacteria during spraying, using Andersen air samplers to measure droplet size, all glass impingers to measure total population, and Ranier slit samplers to measure populations per unit time. Extensive measurements of weather conditions were also taken. Petri dishes with highly selective medium were placed at 148 locations from 1 to 30 meters from the edge of the plot. Pots of sterile bean plants were also placed around the plot, and checked for the presence of recombinant bacteria. Weekly or biweekly measurements for the presence of recombinant bacteria were taken of vegetation and soil around the plot, water taken from a canal near the plot, and on insects collected around the plot.

  After the end of the experiment, all vegetative material, including potato tubers, small tubers and visible roots, were removed and steam sterilized. Plant tissue on the plot was checked in the following year for strains of ice-minus.

- Professor Eric Triplett of the University of Wisconsin conducted a field release of recombinant Rhizobium leguminosarum. The

bacteria were released in July 1990. Nodule occupancy tests were done to check for the presence of recombinant bacteria. High inoculation plots were checked for horizontal and vertical dispersal. Because no bacteria were found in high inoculation plots, low inoculation plots were not checked for horizontal or vertical dispersal. In addition, border rows of clover that were not inoculated with the recombinant bacteria were planted around the plot, and the nodules of these plants were checked for the presence of recombinant bacteria. Because no spread was observed, there were no containment procedures undertaken.

- Professor Donald Phillips of the University of California at Davis has conducted a field release of Rhizobium japonicum. He monitored surface water runoff and checked for nodule occupancy, but did not do air monitoring or sampling (Phillips 1991).

- Dr. Jennifer Parke of the University of Wisconsin has conducted a field release of genetically engineered Pseudomonas fluorescens. The lac ZY marker was used to monitor the fate of the experimental microorganisms on the roots of pea plants and on the roots of adjacent plants. Lateral movement was measured by buffer rows spaced 3 feet apart and monitored in the soil after harvest. After termination of the experiment the organism was found to decline to very low levels and could require long term monitoring. Vertical dispersal also was observed on the roots. Vertical dispersal in the soil was not monitored, nor was airborne migration (Parke 1991).

2. Impact of Regulations on Monitoring Practices

Regulations may in some cases require additional monitoring, control, and containment procedures in order to protect the environment and ensure worker safety that researchers consider costly and unnecessary to the goal of the scientific experiment. The following studies illustrate the concerns of researchers:

- Dr. Steve Lommel of North Carolina State University developed controls for a pilot release in North Carolina. Because these controls were the first developed in the state, they are now used as a model for both the public and private sectors. The procedures developed for control and monitoring were very conservative according to Lommel, because they incorporated suggestions from the Environmental Defense Fund and the Audubon Society. He felt that the procedures could be streamlined to include only those tests that are absolutely necessary to determine control and containment, but he thought that this was unlikely to occur (Lommel 1991).

- Professor Donald Phillips at the University of California at Davis has done field testing in the past, but left the field because regulatory stipulations were too costly (Phillips 1991).

- Professor Eric Triplett at the University of Wisconsin stated that tests of horizontal or vertical dispersal as well as border row nodules tests are required for monitoring, but not for scientific control. Thus, the only test that would have been performed in the absence of regulatory monitoring requirements would have been tests of nodule occupancy of the legume. The workload resulting from the required monitoring procedures was approximately three times that of the scientific requirements (Triplett 1991).

- Recent field test of Rhizobium conducted under EPA authority under TSCA demonstrate that additional monitoring regulations were not imposed (OPTS 1991).

3. Costs for Monitoring, Containment, and Control of Field Releases

The costs for monitoring, containment, and control of field releases is often the most costly part of the experiment. In general, the major cost is attributable to labor (e.g., field hands required to prepare the planting beds and buffer zones). Usually at least one highly trained person, such as a post doctorate, is required for monitoring. In addition, a technician may also be required. Costs can range from $30,000 per year to $600,000 per year. Overall research costs for a typical soil microbial product average from $500,000 to $3 million a year for a five year project (Glass 1991). Initial field releases, releases of organisms whose behavior is uncertain, and a need for more sensitive testing media would be more expensive than would have been subsequent releases, releases of well characterized organisms, and the use of less sensitive testing media.

- Dr. Rich LaMar stated that the costs associated with monitoring, control, and containment result from both capital and labor costs. Labor would be the more costly of the two requirements. He estimated that an additional technical person would be needed to assess monitoring and control procedures. He also stated that they would need separate high performance liquid chromatography apparatus for this work (LaMar 1991).

- According to Dr. Ellis Kline, initial field tests required $600,000 for monitoring and control to closely examine the growth and dispersion of the microorganism and its effect on the environment and human health and safety. Due to the detailed examination for the first field test, and knowledge gained about the microorganism that was released, the second field test was less expensive, costing approximately $60,000 (Kline 1991).

- According to Professor Eric Triplett, costs for monitoring and containment totaled $30,000, approximately 90 percent of which was labor. Costs were low because the bacteria died back and could not be detected after a time, and monitoring was limited to high inoculation plots since the test organisms remained localized. Professor Triplett was planning field releases for the following year and estimating that costs and procedures would be the same as for the above experiment (Triplett 1991).

- According to Dr. Steven Lindow, before the release of the recombinant bacteria, laboratory and greenhouse tests were done to document their safety in regards to human health and the environment. This prior documentation required approximately $100,000 over several years. For the actual field release, monitoring costs amounted to approximately $100,000 a year, most of which were labor costs. However, because this was the first approved FIFRA field release, these costs do not reflect current costs of monitoring and containment, which would be less (Lindow 1991).

- Dr. Jennifer Parke reported that monitoring and control activities were extremely expensive for the field release of the microorganism being studied. These activities account for $42,000 in costs per field season. The costs are attributable to salary of a doctoral researcher, a technician, and an expensive growth medium. She expected these costs to be the same for the duration of the field release experiment, which could range from 2-5 years (Parke 1991).

- According to Professor Donald Phillips, an experiment involving Rhizobium japonicum required one week of technician time to test for traits, and one week of technician time to plant the crop. This experiment was conducted in 1979 and 1980 and was not subject to regulation (Phillips 1991).

- Dr. Steven Lommel felt that testing procedures used for field releases were extremely thorough and that some unnecessary tests were performed. He estimated that procedures could be streamlined over time, resulting in a $10,000 drop in control and monitoring costs. Because the procedures that were developed for Lommel's release are being used as the current model, however, the state may not allow elimination of extraneous control procedures. To the private sector he felt that this amount would not be considerable, however, he asserted that in the public sector this extra amount could discourage some projects (Lommel 1991).

All of the individuals contacted agreed that the most significant category of cost is labor. One contact stated that the amount of monitoring and containment procedures performed for regulatory reasons as compared to procedures performed for product development depends upon the type of project. For agricultural-type environmental applications, a company often needs very

similar information to information that EPA desires. For other types of environmental applications, a company usually has little interest in the information that EPA desires (D. Glass Assoc. 1991). One company spokesman attributed 20 percent of the total monitoring costs for environmental application field tests to regulations and the anticipation of regulations (Envirogen 1991b).

4. Academic Research on Microorganisms not Involving Field Testing or Environmental Releases

Much of university research in biotechnology does not include environmental applications or field tests of microorganisms in the environment. Microorganisms are often studied in the laboratory in "clean systems" also known as microcosms or mesocosms (where intact pieces from the ecosystem such as soil, water, or wood are brought into the laboratory to mimic the field environment) (National Research Council 1989). Some studies are also conducted in contained greenhouses.

In most cases, these experiments follow NIH guidelines and adhere to proper laboratory procedures for monitoring and containment. Some scientists suggest that their research may lend itself to environmental releases in a few years (Verma 1991), but others state that they plan to do only basic research in the future (Stacey 1991).

Although laboratory experiments do not require the types of monitoring and containment procedures that are required in the field, some basic research is directed towards the development of techniques to monitor or contain a microorganism for a field test or commercial release. Grant funding for this research comes from various sources, including private companies, university funds, NIH, and other state or federal agencies.